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THE MONOSYNAPTIC EXCITATORY CONNECTIONS OF SINGLE TRIGEMINAL INTERNEURONES TO THE V MOTOR NUCLEUS OF THE RAT

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SUMMARY

1. We have used the extracellular spike-triggered averaging method to identify a population of trigeminal interneurones that make monosynaptic connections within the V motor nucleus. The experiments were performed on rats anaesthetized with pentobarbitone (60 mg/kg I.V.; supplementary doses given as necessary to maintain a deep level of anaesthesia).

2. A tungsten microelectrode (tip exposure of some 200 μm) was inserted into the masseter motoneurone pool to allow recording of extracellular activity. A glass electrode filled with DL-homocysteic acid was used to make simultaneous extracellular recordings of the firing of single neurones in the region immediately caudal to the motor nucleus.

3. Fifty-eight out of 166 interneurones tested gave unitary extracellular fields in the motor nucleus. The responses consisted of a terminal spike (presynaptic spike) followed by a negative field of duration approximately 3 ms and amplitude 0·4–10·8 μV. The mean latency between the positive peak of the terminal spike and the onset of the field (synaptic delay) was 0·43 ms (S.D. = 0·10 ms), and the mean latency from the onset of the interneurone spike to the positive peak of the presynaptic spike was 0·35 ms (S.D. = 0·22 ms). Thus the interneurones project directly to the motor nucleus where they then make monosynaptic connections.

4. The negative extracellular fields were taken to reflect an excitatory synaptic input onto neurones within the motor nucleus. The fields were of maximum amplitude at the point of maximum masseter motoneurone antidromic field, suggesting that the connection may be onto elevator motoneurones.

5. The location of the interneurone appeared to the main factor governing the likelihood of obtaining a field. Interneurones located more than 400 μm from the caudal border of the motor nucleus rarely produced fields whereas most of those located within this distance gave fields. This pattern of distribution is strikingly similar to that of trigeminal interneurones labelled by the transneuronal transport of wheatgerm agglutinin–horseradish peroxidase after an intramuscular injection of the tracer into the masseter muscle. We suggest that this provides electrophysiological support for the suggestion that the tracer does indeed label interneurones by means of retrograde transsynaptic transport.

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INTRODUCTION

Spike-triggered averaging techniques provide a powerful tool for unravelling the rules governing the organization of connections between neurones. Although for motoneurones much has been revealed by these methods of the organization of the connections of some inputs, notably those from muscle spindle afferents (reviewed in Henneman & Mendell, 1981), relatively little is known about how the monosynaptic connections of (last-order) interneurones on to motoneurones are organized even though this is the route by which virtually all reflex inputs and some descending ones act on motoneurones. An important source of difficulty is simply in recognizing where such interneurones are located.

One recent solution to this problem has been to use the horseradish peroxidase-conjugated form of wheatgerm agglutinin (WGA–HRP) as a tracer. Injections of WGA–HRP into either a muscle nerve or muscle result in labelling of interneurones by means of retrograde transneuronal transport of the tracer (Harrison, Hultborn, Jankowska, Katz, Storai & Zytnicki, 1984; Harrison, Jankowska & Zytnicki, 1986). Indeed the starting point for this study was the observation that trigeminal interneurones could also be labelled after injections of WGA–HRP into the masseter muscle (Appenteng, Girdlestone & Holden, 1985; Appenteng & Girdlestone, 1987). The interneurones labelled appeared to form a shell around the lateral and caudal borders of the V motor nucleus in areas corresponding to the medialmost part of the V main sensory nucleus and a region starting at the caudal border of the V motor nucleus and extending 500 μm caudal from this (Appenteng & Girdlestone, 1987). It was suggested that the interneurones were labelled as a result of retrograde transneuronal transport of tracer from motoneurones. However, the arguments were necessarily indirect given the absence of a clear separation between afferent and efferent fibres in cranial muscle nerves. Thus the immediate aims of the present study were to determine electrophysiologically whether neurones in the region just caudal to the V motor nucleus make monosynaptic connections within the motor nucleus and if so to determine the sign of this connection.

The technique of choice in this situation is the extracellular spike-triggered averaging method because it allows one to readily determine if a single neurone makes a synaptic connection onto a target population of neurones (Appenteng, O'Donovan, Somjen, Stephens & Taylor, 1978; Taylor, Stephens, Somjen, Appenteng & O'Donovan, 1978). We have used this method to reveal a monosynaptic excitatory connection from interneurones in the area described above onto neurones in the V motor nucleus. An abstract of the work has appeared (Appenteng, Bonte & Moore, 1989).

METHODS

Surgical preparation. This was essentially as described earlier by Appenteng, Donga & Williams (1985), the major difference being that anaesthesia was maintained by I.V. infusions of pentobarbitone (initial dose = 60 mg/kg). In brief, rats in the weight range 200–250 g were initially anaesthetized with halothane in oxygen. A femoral venous catheter was inserted and further anaesthesia maintained by I.V. infusions of pentobarbitone. The trachea was cannulated and blood pressure monitored by a cannula in the femoral artery. The left masseter nerve was exposed in-continuity and a pair of silver wires placed around the nerve to allow electrical stimulation. Animals were then transferred to a stereotaxic holder and their heads held as described

by Pellegrino, Pellegrino & Cushman (1981). A hole was drilled in the cranium to allow access to the motor nucleus. Animals were paralysed with gallamine triethiodide and artificially ventilated for the duration of experiment. A bilateral pneumothorax was performed and end-tidal carbon dioxide levels monitored. The animals were maintained deeply anaesthetized throughout all stages of the experiment. The criterion used was that a noxious paw-pinch should elicit no change in blood pressure and under these conditions there was no flexion withdrawal reflex in the unparalysed animal.

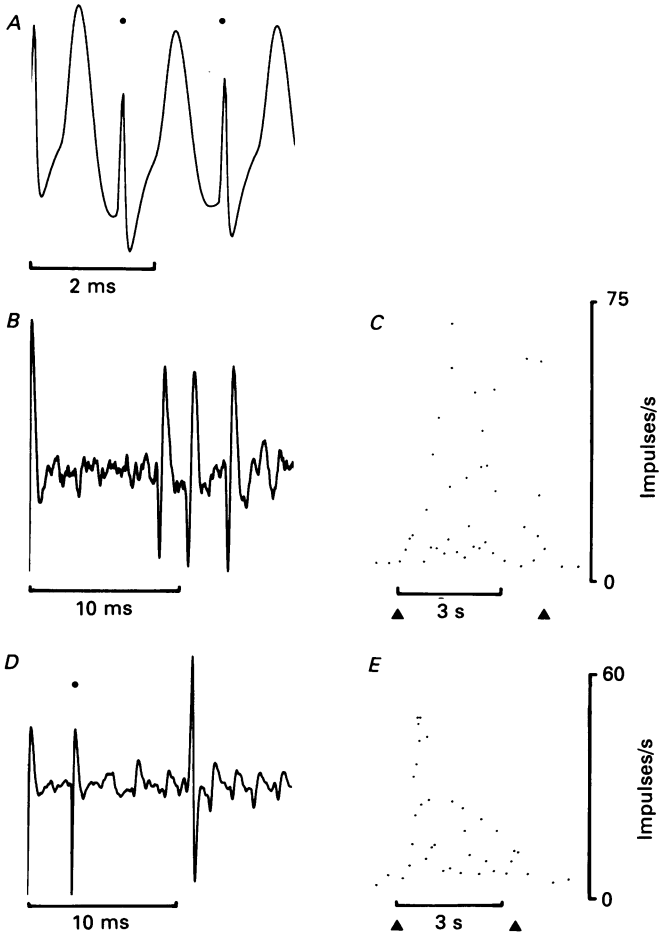

Fig. 1. Identification of a masseter muscle afferent (*A*) and interneurones (*B–E*). Left-hand panels show responses to electrical stimulation of the masseter nerve and right-hand panels the response to DLH application. The afferent was activated at short latency (0·4 ms) and followed 1:1 a train of stimulus pulses spaced at intervals of 1·3 ms (●, onset of stimulus pulses.). It was not activated by DLH application (not shown). Interneurones either fired repetitively to a single shock of the masseter nerve (*B*) or fired in response to two (*D*) or more closely spaced shocks of the nerve. Both interneurones fired to DLH application (*C* and *E*; arrow-heads mark onset and termination of ionophoresis), so identifying the recordings as being from a somatic site.

Electrodes. Extracellular recordings from single neurones were made with glass microelectrodes filled with a 1 M solution of DL-homocysteic acid (DLH; pH = 8·0). The electrodes were bevelled to resistances of 12–15 MΩ and could all pass at least 10 nA of current in either direction without showing rectification. Extracellular recordings were made in the V motor nucleus using glass-

coated tungsten microelectrodes with approximately 100–200 μm of their tip exposed and a diameter of 20–50 μm at the point where the insulation ceased.

Protocol. The protocol adopted was shaped by the need to locate the two electrode tips within 700 μm of each other in structures some 8 mm below the surface of the cerebral cortex. The strategy adopted was first to locate the middle of the masseter motoneurone pool with the DLH-filled electrode. This was signalled by the presence of an antidromic field of amplitude 1·5–2·0 mV. The electrode was then withdrawn and positioned 400–500 μm more caudally so as to place it immediately caudal to the motor nucleus. There were no distinct fields in this area and so the only

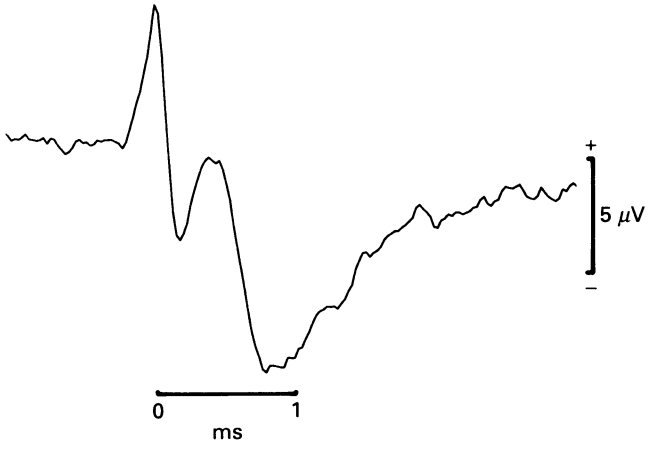

Fig. 2. Unitary extracellular field generated by a masseter spindle afferent (identified in Fig. 1*A*) within the V motor nucleus. Start of time bar marks onset of afferent trigger spike; 480 sweeps used to derive the average.

sure means of ascertaining the electrode position was by reference to the position where the maximum antidromic field was obtained. The tungsten electrode was then inserted into the brain at co-ordinates that would place it in the rostral part of the motor nucleus. It was then moved more caudally into the centre of the masseter motoneurone pool which in this case was signalled by the presence of a 1 mV antidromic field. It was then left in this track for the remainder of the experiment.

Unit identification. Single units were isolated with the DLH electrode and their projection to the motor nucleus studied by constructing perispike averages. Neurones that lay outside the motor nucleus or the mesencephalic nucleus (areas known to contain motoneurones and afferents respectively) were assumed to be interneurones for the purposes of this study. Thus on this basis any somatic recordings obtained from outside these areas must by definition be from interneurones. The search strategy used was to track with a continuous low (less than 0·2 nA) ejection of DLH and to electrically stimulate the masseter nerve at 1 s intervals. Ionophoretic application of DLH was then used to distinguish between somatic and axonal recordings. The latter were assumed to be fibres of passage and so were ignored. Somatic recordings were then further tested to determine the pattern of inputs on to them using both natural (non-nociceptive) stimulation applied to the mandibular and maxillary areas and also electrical stimulation of the masseter nerve (Fig. 1). Almost all neurones could be activated by some combination of these stimuli but the few that could not were excluded from further consideration. Recordings were also made from spindle afferent axons within the mesencephalic tract. These were identified by the criteria of increased firing on gentle muscle probing and increased firing on jaw opening.

All data was recorded on-line using a C.E.D. 1401 interface (Cambridge Electronic Devices) set to sample at 40 kHz for perispike averaging and at 60 kHz for poststimulus averaging. Extracellular activity recorded by the tungsten electrode was bandpass filtered between 55 Hz and 5 kHz and the unitary activity recorded by the glass electrode filtered as appropriate. Electrode tracts were identified using standard histological techniques after the completion of each experiment.

RESULTS

Spindle afferent fields

Our first step was to determine if we could use the extracellular spike-triggered averaging method to reveal a previously known connection to the motor nucleus. The obvious such candidate is the monosynaptic connection formed by elevator spindle afferents for which there is now specific morphological evidence in the rat but as yet no specific electrophysiological evidence (Appenteng *et al.* 1985; Dessem & Taylor, 1988). Twelve spindle afferents were studied and all generated unitary extracellular fields within the motor nucleus. These consisted of an initial biphasic spike followed after an interval by a negative-going field potential (Fig. 2; see also Appenteng *et al.* 1978; Taylor *et al.* 1978). The biphasic spike can be ascribed to spread of the action

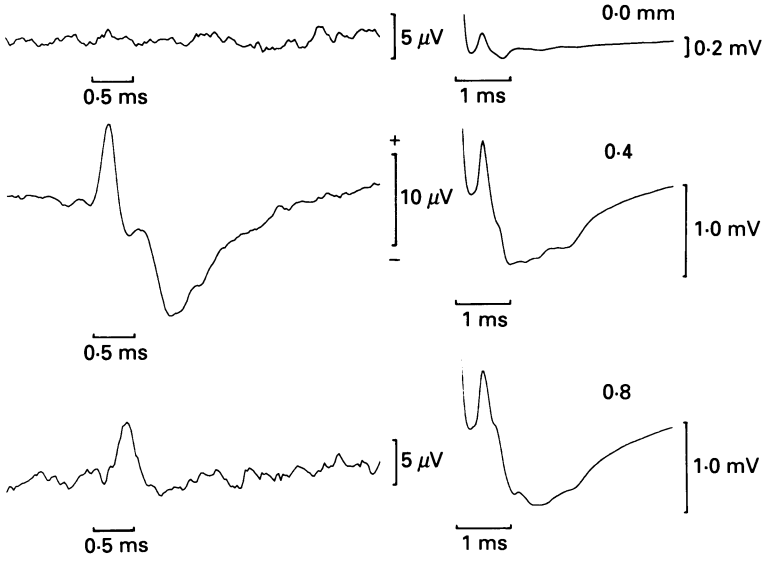

Fig. 3. Traces on left show unitary field generated by a masseter spindle afferent and on the right the masseter nerve antidromic field at the same depths. Note that the afferent field is localized to the dorsal part of the motor nucleus. Time bars on left mark onset of afferent triggering spike. Number of sweeps used: unitary fields, 1063 sweeps in top trace, 535 in middle and 832 in bottom trace. Eight sweeps used for all antidromic fields.

potential along the collateral branches of the axon and the negative field to the inward flow of synaptic current into postsynaptic elements. The latency from the positive peak of the biphasic spike (presynaptic spike of Katz & Miledi, 1965) to the onset of the negative field represents the mean delay across all the synaptic terminals of the afferent within the motor nucleus. The mean value obtained for the afferent sample was 0·42 ms (S.D. = 0·11 ms) and so points to a monosynaptic connection. The latency from the onset of the triggering spike to the positive peak of the presynaptic spike would be expected to provide an estimate of the maximum conduction time

between the recording sites. However, a source of ambiguity in the case of spindle afferents is that an action potential from the periphery would arrive first at the motor nucleus before proceeding to other sites (Appenteng *et al.* 1985). Thus the apparent conduction time would depend on the relative conduction velocities along the axonal branch to the recording site and on the conduction velocity along the terminal collaterals in the motor nucleus (Appenteng *et al.* 1978). Fields generated by spindle afferents were restricted to the confines of the motor nucleus and as predicted by the morphological data were of maximal amplitude in its dorsal part (Fig. 3). The mean amplitude of these fields was 5·3 μV (S.D. = 3·9 μV) and that of the presynaptic spikes was 6·9 μV (S.D. = 4·9 μV).

Interneurone fields

Having established the adequacy of our technique we then turned to examining the connections of interneurones. Figure 4*A* and *C* shows examples of the fields generated by single interneurones within the motor nucleus. They can be seen to be identical in form and polarity to those generated by muscle spindle afferents. On occasions clear fields were seen after as few as ten sweeps of the averager but in general between 50 and 100 sweeps were required. Additional sweeps were then accepted, largely to further increase the signal-to-noise ratio.

Figure 4*B* and *D* shows responses obtained when averaging at the same points in the motor nucleus as in Fig. 4*A* and *C* respectively but this time triggering off interneurones found no more than 100 μm away in the same electrode tracks. The absence of any semblance of a response in Fig. 4*B* and *D* suggests that the method can be used to reveal differences in the synaptic connections of interneurones. This assumes that the method is reliable and in support we would point to the fact that it can be used to consistently demonstrate a known connection, namely the projection of muscle spindle afferents into the motor nucleus.

We have studied the projections of 166 interneurones to the motor nucleus and obtained unitary extracellular fields of negative polarity from fifty-eight of them. Units that consistently fired bursts of spikes at high frequency invariably generated a series of fields and this is illustrated in Fig. 5 for an interneurone that fired in triplets. The spike amplitudes remained constant throughout the average but the interval between spikes in a burst varied. The first two spikes in the bursts produced fields but not the third spike. The most striking difference in the responses concerned the relative amplitudes of the presynaptic spikes, but there were also clear differences in both the time from field onset to the point of maximum amplitude and in the amplitudes of the fields themselves. The differences may arise from fluctuations in the timing of the second spike after the first and as such would indicate that the amplitude of the averaged presynaptic spike is remarkably sensitive to such variation. Such variation would also be expected in situations where the firing of the triggering spike was synchronized to that of another neurone.

All the negative fields generated by interneurones were accompanied by sharp and prominent presynaptic spikes. Indeed the mean amplitude of these (3·6 μV; S.D. = 2·7 μV) was similar to that of the fields themselves (3·19 μV; S.D. = 2·13 μV; $n = 46$). Neither of these values were statistically different from the figures obtained for spindle afferents ($P = 0{\cdot}5$ in each case). The mean conduction time from the interneurone somata to the motor nucleus was 0·35 ms (range = 0·06–0·96 ms;

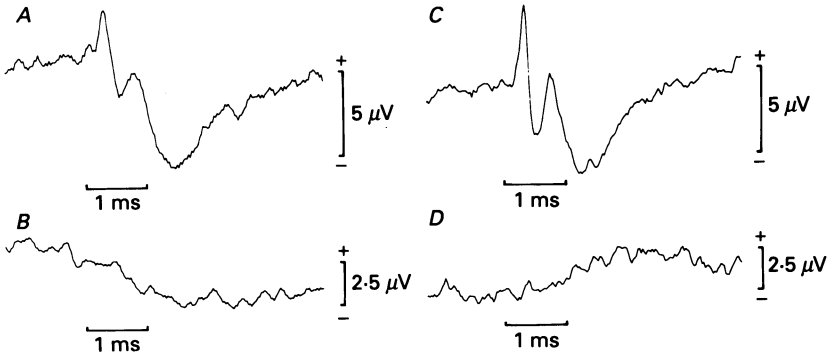

Fig. 4. Responses produced by pairs of interneurones (*A*, *B* and *C*, *D*) at the same point in the motor nucleus in two different experiments. In each case the interneurone pairs were recorded in the same electrode track and within 50 μm of each other. Time bars mark onset of triggering spikes. Number of sweeps used: $A = 861$; $B = 1000$; $C = 620$; and $D = 602$. Receptive fields of interneurones: *A*, masseter muscle (latency = 3·6 ms), upper incisor and skin on both lips; *B*, masseter muscle only (latency = 4 ms) but no response to jaw opening; *C*, masseter muscle (latency = 12 ms), tongue and both lips; *D*, masseter muscle (latency = 8·74 ms), tongue and lower incisor.

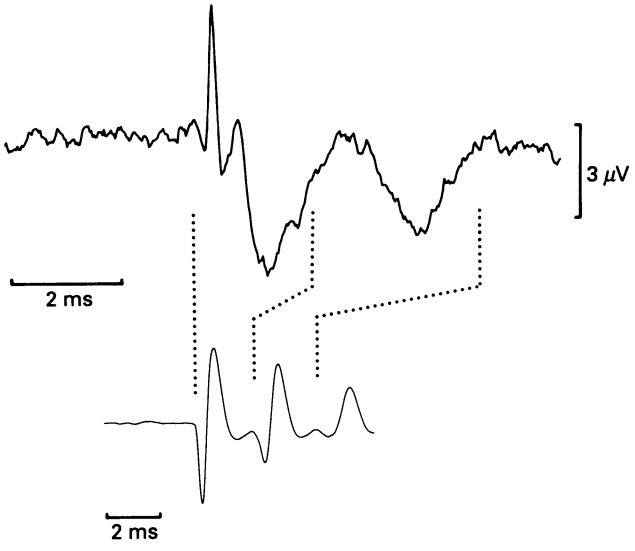

Fig. 5. Multiple fields generated by a repetitively firing interneurone. Top trace shows unitary fields (915 sweeps) and bottom trace the interneurone firing (fifty sweeps). Dotted lines mark onset of interneurone spikes. The interneurone fired in bursts (duration 10–12 ms). The first spike in a burst provided the trigger for the averager which was set to sample for 20 ms (5 ms before and 15 ms after trigger). Interneurone activated by pressure to upper and lower incisors, tongue and both lips.

S.D. = 0·22 ms; $n = 46$). In all cases latencies were measured from the onset (i.e. the initial deflection from the baseline) of the interneurone spike to the positive peak of the presynaptic spike in the motor nucleus. The synaptic delay was measured as the latency between the positive peak of the presynaptic spike and the onset of the field.

The mean value obtained was 0·43 ms (range = 0·23–0·59 ms; S.D. = 0·10 ms; $n = 42$). These figures indicate that the axons of interneurones travel directly to the motor nucleus where they make synaptic connections.

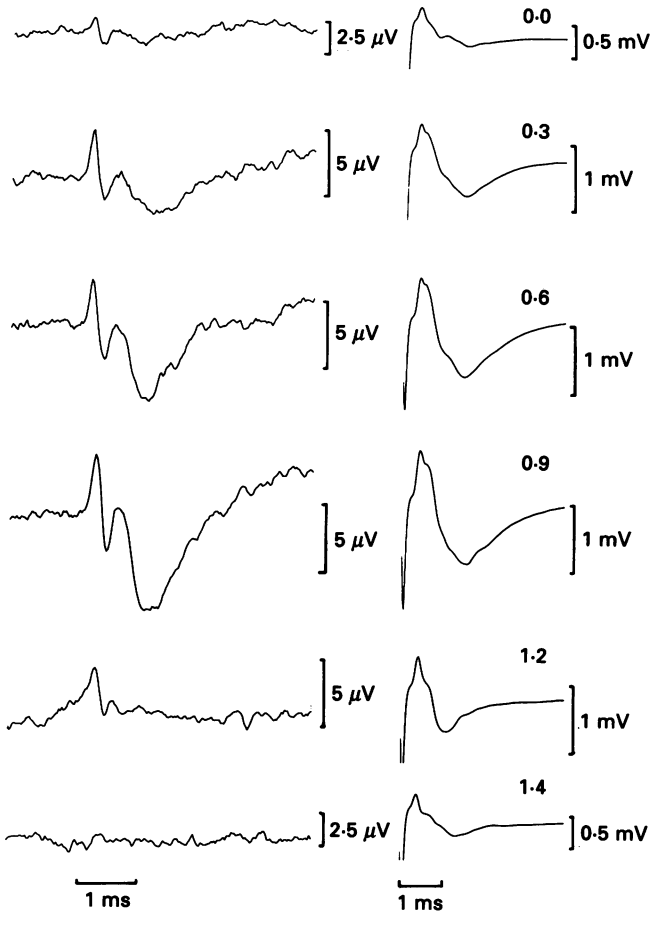

Fig. 6. Systematic mapping of the field generated by an interneurone at different depths in the motor nucleus. The unitary fields are shown on the left (602–736 sweeps) and the antidromic masseter-evoked fields on the right. Note that the unitary fields do not reverse in sign through the nucleus and that they are maximal at the depth of maximum antidromic field. Interneurone activated by inputs from hairs on both lips.

The sign of this connection was assessed by systematically recording the fields generated by single interneurones at varying depths in the motor nucleus. An example of this is shown in Fig. 6 where both the unitary field and the antidromic field were recorded at depths starting just above the motor nucleus (top traces) and finishing just ventral to it (bottom traces). The changes in both the form and amplitude of the unitary field seen between the arbitary zero depth and a depth of 0·9 mm are fully consistent with approach of the electrode to a current sink. The field does not reverse in sign more ventrally but simply disappears. It follows that the

negative fields generated by the interneurone represent an excitatory input onto cells that are located near the point of maximum antidromic field.

An additional thirteen interneurones generated only a presynaptic spike at depths in the motor nucleus where other interneurones gave fields. These presynaptic spikes were quite distinct, ranging in amplitude up to 19 μV (mean = 5·82 μV). Our

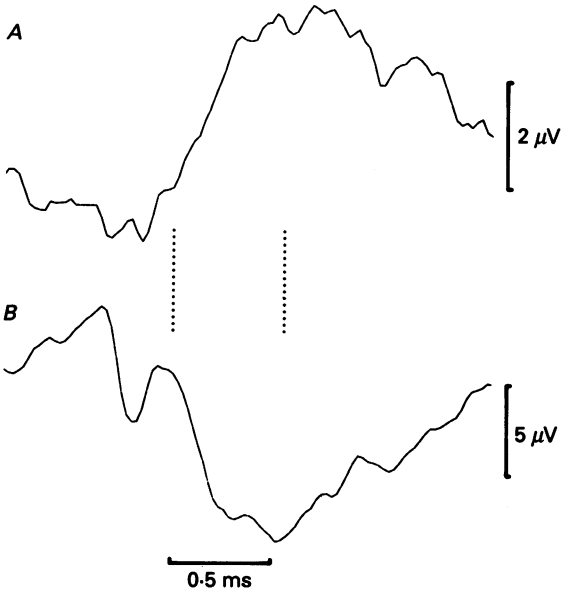

Fig. 7. Comparison of a positive (*A*; 1000 sweeps) and negative (*B*; 502 sweeps) unitary field recorded at the same point in the motor nucleus from two interneurones obtained in the same track. The dotted lines mark onset and peak of the two fields and serve to emphasize that the fields appear to be inverted versions of each other. Note the absence of a presynaptic spike in *A*. Receptive fields of interneurones: *A*, skin on upper lip. *B*, masseter muscle (latency 2·8 ms), tongue, upper and lower incisors, both lips and skin over cheek.

assumption was that these axons terminated in a different part of the motor nucleus or that they were coursing through the nucleus on their way elsewhere. We could not distinguish between these possibilities because it was not possible to record fields from an interneurone in more than one track through the nucleus due to the proximity of the two electrodes.

Four interneurones generated positive fields in the motor nucleus. None of these fields was preceded by a prominent presynaptic spike. In two cases the fields commenced either before or were coincident with the onset of the triggering spikes and so we assumed them to be the consequence of synchronization between the triggering neurone and another unidentified neurone (see Kirkwood & Sears, 1980). In the remaining two cases the fields occurred at latencies of 1·2 and 0·88 ms (Fig. 7*A*) after the onset of the triggering spike and appeared to be mirror images of the negative fields more commonly recorded (Fig. 7*B*). The polarity of the fields suggests an inhibitory connection while the latency values cited are compatible with direct conduction of activity to the motor nucleus followed by monosynaptic activation of

neurones. The absence of clear presynaptic spikes suggests that the fields are not unitary and so may result in part from synchronization between the triggering neurone and another neurone. None the less the very presence of these positive fields suggests that inhibitory connections can be detected with the extracellular spike-triggered averaging method and therefore the fact that they were so rare in our material implies that few of the interneurones in the area examined make such connections within the masseter motoneurone pool. An alternative possibility that we cannot fully exclude is that the positive fields represent outward current from remote excitation.

Receptive fields and location of interneurones

Ten of the thirteen interneurones generating only presynaptic spikes were activated by a combination of afferent input from the masseter muscle and intraoral tissues ($n = 7$) or additionally by input from cutaneous tissue in the trigeminal area as well ($n = 3$). Two interneurones were activated by cutaneous input only and one by input from the masseter muscle. Just under half of the fifty-eight interneurones which gave negative fields were activated by afferent input from only one source. Thirteen were activated by input from intraoral tissues, eight from masseter and four from skin. The remaining interneurones were activated by a combination of inputs from either skin and intraoral tissues ($n = 15$), muscle and intraoral ($n = 10$), muscle and skin ($n = 4$), and muscle, skin and intraoral ($n = 4$). We were able to localize the source of the intraoral input from neurones that generated fields. In nineteen instances the input was from the lips, the incisors in fifteen, the tongue in eight and from all three in eight cases. In contrast we were rarely able to localize the receptive fields of neurones activated by the masseter nerve. However, two of the neurones activated by just the masseter nerve showed an increased firing during jaw opening, perhaps suggesting that they received an input from spindle afferents. Six other neurones showed similar behaviour but these did not produce fields. In all cases the natural stimuli applied were judged to be non-noxious. Indeed noxious stimuli such as a firm pinch of the nose could produce a reduction in discharge of units that persisted after removal of the stimulus.

All units that produced fields were located in a discrete area close to the caudal borders of the motor nucleus. We initially established this in a preliminary qualitative study where we made recordings from interneurones lying up to 2 mm caudal to the motor nucleus. Sixty-four interneurones (not included in the data cited above) were recorded and fields were obtained from fourteen of them. Units that produced fields tended to lie in clusters and furthermore these were mostly within 400 μm of the caudal borders of the motor nucleus. All of the fifty-eight units that produced negative fields in the main part of the study were also located within 400 μm of the caudal border of the nucleus. We verified this histologically at the termination of experiments but during the experiments we were guided by how far caudal the electrode track was to the position of maximum antidromic field (see Methods). Only one of the sixty-four interneurones that lay more than 900 μm from the point of maximum antidromic field produced a unitary field. This corresponded to a position more than 400 μm caudal to the nucleus. In contrast fifty-two out of seventy-nine interneurones that lay within 400 μm of the motor nucleus produced

fields. The proportion of interneurones giving fields increased with rostral advancement of the electrode up to the point where recordings could be obtained from masseter motoneurones using the criteria of Appenteng *et al.* (1985). The most fruitful tracks were those made no more than 200 μm away from this point and in these tracks virtually all neurones produced fields.

DISCUSSION

Our main finding is that interneurones in the region immediately caudal to the V motor nucleus make monosynaptic excitatory connections within the motor nucleus. The interneurones are located precisely where neurones labelled by the transneuronal transport of WGA–HRP had been reported (Appenteng & Girdlestone, 1987) and so we suggest that this provides electrophysiological evidence to support the suggestion that the tracer does indeed label interneurones by retrograde transneuronal transport of tracer from motoneurones into last-order interneurones. Thus the two studies provide the first specific identification of a population of last-order trigeminal interneurones and, more generally, a rare instance of a group of excitatory last-order interneurones (see also Cavallari, Edgley & Jankowska, 1987). Furthermore the interneurones described here appear to be nearly all excitatory in the synaptic effects that they elicit. Thus it may be possible to explore the pharmacology of this connection using population techniques (e.g. see Appenteng & Saha, 1988).

The extracellular variant of the spike-triggered averaging technique has not proved to be as popular as the sucrose-gap techniques for examining the connections of single neurones onto a population of motoneurones (Luscher, Ruenzel, Fetz & Henneman, 1979; Brink, Harrison, Jankowska, McCrea & Skoog, 1983; Cavallari *et al.* 1987). The main advantage of the sucrose-gap method is that both the pre- and postsynaptic elements are identified. However, the method cannot be applied to cranial motor systems because afferent and efferent fibres are not segregated in structures equivalent to dorsal and ventral roots, and in addition the recording sites would be at considerable distances from the relevant motor nuclei and so the signals would be grossly attenuated. The extracellular spike-triggered averaging method is perhaps of more general use and indeed has recently been used to reveal the connections of muscle afferents in the cat hindlimb (Collins, Mendell & Munson, 1986). A specific advantage of the method is that the presynaptic spikes are clearly recorded and for the reasons already outlined in connection with Fig. 5 provide a simple means of assessing if a response is affected by synchronization of presynaptic neurones. Thus our view is that the spike-triggered averaging method can reliably be used to reveal the unitary projections of interneurones.

This stance differs from that adopted by Brink *et al.* (1983) who used the sucrose-gap method to identify interneurones with a group I input and an axon collateral ascending to L4. They also evoked interneuronal firing by ionophoretic application of DLH (or glutamate) but their experience was that sixteen out of twenty-one interneuronal responses were preceded by a relatively large 'average common excitation potential' (ACE potential of Kirkwood & Sears, 1978; Kirkwood & Sears, 1980) which they attributed to inputs that were common to both the pre- and postsynaptic neurones. They used this to point out the difficulty of being confident

that responses obtained by the spike-triggered averaging method are evoked by the interneurone under test as opposed to being simply correlated with their activity. However ACE potentials were not a feature of the data reported by Cavallari *et al.* (1987) in their study on interneurones in the L4 segment that project to hindlimb muscles. The ACE potentials seen by Brink *et al.* (1983) are presumably then a consequence of a specific set of connections found on group I interneurones but not on either the L4 interneurones of Cavallari *et al.* (1987) or those described by us. A further difference between the two spinal studies concerns the presence of presynaptic spikes. It may be significant that a presynaptic spike is only evident in one of the four unitary responses shown by Brink *et al.* (1983) but is present in all the four responses shown by Cavallari *et al.* (1987). The problems of assessing if a sucrose-gap record represents unitary activity are further complicated by the fact that the rise time of the recorded events is slowed more than would be expected simply as a result of passive attenuation of the signal along the motor axons (Brink *et al.* 1983). Therefore one cannot use a 'short' rise time as a criterion for assessing if a response is unitary (Davies, Kirkwood & Sears, 1985) and the presence or absence of a presynaptic spike assumes added importance.

The majority of the trigeminal interneurones were activated by afferent inputs from the intraoral tissues. Afferents from this source are known to be predominantly active during the closure phase of chewing movements (Appenteng, Lund & Seguin, 1982). Therefore one would expect the afferent input to increase the excitability of the interneurones during this phase of the movement and this would then enhance the activity of the jaw-closing muscles themselves. On this view the interneurones would provide part of the normal drive for the closure phase of chewing movements because they are part of a positive feedback loop. The underlying level of interneuronal activity could be set by central pathways and in this way they could regulate the potency of the feedback onto elevator motoneurones.

On the surface the above scheme would appear to run counter to the classic belief that the reflex effects elicited by intraoral afferents are an inhibition of the jaw elevators and, depending on the modalities activated, an excitation of the openers (Sherrington, 1917; Dessem, Iyadurai & Taylor, 1988). The strongest challenge to this view has come from work by Lavinge, Kim, Valiquette & Lund (1987) on the effects of obstructing the closure phase of cortically induced masticatory movements in anaesthetized rabbits. The observation was that there was a reflex jaw opening when the teeth first encountered the resistance (a steel ball) to closure. However, the reflex was absent in subsequent cycles and instead there was an increase in the amplitude and duration of the EMG activity of all jaw-closer muscles. Their suggestion was that input from periodontal afferents could provide positive feedback to the jaw-closing muscles during mastication and this finds support in our work.

The apparent differences between Sherrington's concept and the emerging one are readily reconcilable by taking into account the rather complex reflex effects reported on electrical stimulation of the lingual and inferior alveolar nerves. A single shock to the lingual nerve elicits an IPSP, followed by a depolarization and then a subsequent hyperpolarization in masseter motoneurones (Goldberg & Nakamura, 1968). The extent of the depolarization varies but in some motoneurones it is sufficient to trigger a spike. Conditioning stimuli applied to the inferior alveolar nerve cause an initial

depression, followed by a partial recovery and then a further depression of the monosynaptic reflex from the masseter nerve (Kidokoro, Kubota, Shuto & Sumino, 1968*a*). The early phase of depression is reduced after strychnine while the recovery phase is seen as a period of facilitation, perhaps pointing to the unmasking of an underlying EPSP during the partial recovery phase. Taken together the evidence points to the existence of separate pathways that mediate excitatory and inhibitory effects in elevator motoneurones following activation of intraoral afferents. We have identified the group of excitatory interneurones but it is perhaps worth emphasizing that there is as yet no specific evidence as to where the inhibitory interneurones are located. There is some indirect evidence that these may lie in the supratrigeminal nucleus of the V nerve (Kidokoro, Kuboto, Shuto & Sumino, 1968*b*) but this is clearly an issue that needs to be tackled by the use of signal-averaging methods.

Our suggestion is that the excitatory and inhibitory pathways may be active during mastication in which case one would have the intriguing situation of an afferent modality contributing to the regulation of a movement by virtue of both positive and negative feedback loops. The balance between the two pathways could be modulated by central pathways and in this way afferent activity could then be used in the reflex regulation of the force and duration of the closure phase of normal masticatory movements.

A test of this hypothesis would involve making recordings from the interneurones described in this paper during normal masticatory movements. Such experiments will not be practical until the criteria for identifying the interneurones have been simplified. We have shown that the interneurones are homogeneous in their synaptic actions in the motor nucleus and in addition are discretely localized. The need now is to assess if they are also homogeneous in terms of their projections to other areas as this would provide a further means of identification.

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